

The Delivery of Probiotics and Prebiotics to Infants

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Abstract: A number of studies involving the feeding of probiotics and prebiotics to infants have been published over the last decade. These studies have examined a wide range of health outcomes, including growth and safety, prophylaxis and alleviation of diarrheal disease, reduction in atopic disease, reduction in necrotizing enterocolitis, and reduction in infection of the preterm infant. In addition, these studies have described microbiological alterations observed in response to probiotic and prebiotic feeding. Collectively, the reports demonstrate that probiotics show considerable promise in addressing several health outcomes of significance to both formula-fed and breastfed infants. As quantitative and qualitative differences appear to exist between the microfloras of human-milk fed and formula-fed infants, recent innovations to infant formula have involved the inclusion of probiotics and prebiotics as a means of making the flora of the formula fed infant more similar to that of the breastfed infant. To date, only a few probiotic- and prebiotic-containing infant formulas have been marketed, but as new safety and efficacy data emerge and the regulatory climate becomes more favourable, the number of products is expected to grow.

Key Words: Bifidobacteria, infant, infant formula, lactobacilli, prebiotics, probiotics.

INTRODUCTION

At birth, the gastrointestinal tract is sterile, but it rapidly becomes colonized by microbes associated with the birthing process and the delivery environment. The microflora persists in a dynamic state and undergoes significant changes in the first several weeks of life, but then begins to stabilize until the introduction of solid foods [1-3]. Infant feeding practices appear to have an impact on the gastrointestinal microflora [4-6]. In general, formula feeding results in a more diverse group of microbes in the distal gastrointestinal tract when compared to breastfeeding. Although bifidobacteria are predominant among these microbes, they also include a greater number of facultative anaerobes, bacteroides, and clostridia than found in human-milk fed infants. In contrast, the gastrointestinal microflora of human milk-fed infants appears to be less complex, and tends to harbor a greater number of bifidobacteria than found in formula-fed infants. However, it is of importance to note that forming broad generalizations about the "differences" between formula-fed and human milk-fed infants is difficult because of conflicting data and the presence of rare exceptions to apparent rules.

In this review, we have summarized the major studies involving the use of probiotics and prebiotics in infants (Table 1).

For the purposes of this review, infants have been defined as 12 months of age or younger. In some cases, however, the studies also include children older than one year and/or the period of the study evaluation extends beyond the subjects' first year of life. Although in most of the studies infant formula was used as the vehicle for the administration of the probiotic and prebiotic, some of the

work described in this review involved the delivery of probiotics by capsule, oral rehydration solution (ORS), or other means.

PROBIOTICS AND PREBIOTICS

The word probiotics has taken on a number of definitions and is an evolving term, but essentially describes microbes that are administered with the intention of conferring some health benefit [49]. As components of infant formula, typically-used probiotic bacteria are members of the genera *Lactobacillus*, *Bifidobacterium*, and, to a lesser extent, *Streptococcus*. Bifidobacteria are high G+C, Gram-positive, obligately anaerobic, non-sporeforming, non-motile rods of varied shape, whereas lactobacilli are low G+C, Gram-positive, facultatively anaerobic, non-sporeforming, non-motile rods. Specific organisms used in infant formula include: *Lactobacillus rhamnosus* GG (LGG), *Bifidobacterium lactis*, and *Streptococcus thermophilus*.

The term prebiotic has been used to describe a dietary component that stimulates the growth and/or activity of a selected group of microbes, thereby providing these organisms with a competitive advantage over other bacteria in the ecosystem [49]. By evading digestion and absorption in the proximal regions of the gastrointestinal tract, the prebiotic becomes available as a substrate for the microbes of the distal gastrointestinal tract. The conventional prebiotic is a nondigestible oligosaccharide, and the prototypical prebiotic is fructooligosaccharide (FOS). Several recent trials in infants have been conducted to evaluate the effects of feeding FOS, galactooligosaccharides (GOS), or a combination of the two carbohydrates (Table 1).

MICROBIOLOGICAL OBSERVATIONS

In many of the studies that have involved examination of the use of probiotics and prebiotics in infants, the primary health outcomes have been microbiological in nature [9-11

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Table 1. Major Studies on Use of Probiotics and Prebiotics in Infants and Young Children

Author/Year	Design	Location	N	Age at Enrollment	Health Status	Length of Study	Vehicle	Ref.
Isolauri <i>et al.</i> 1991	Randomized, double blind, placebo-controlled; 3 arms: 1. Control/placebo: fermented-then-pasteurized yogurt 2. Experimental: LGG-fermented milk product 3. Experimental: LGG freeze-dried powder	Tampere University Hospital, Finland	56	4-45 months	Acute diarrhea of <7 days duration with >3 watery stools in previous 24 hours	5 days	Milk product and powder	[7]
Kaila <i>et al.</i> 1992	Randomized, placebo-controlled; 2 arms: 1. Control/placebo-fermented then pasteurized yogurt 2. Experimental: LGG-fermented milk	Tampere University Hospital, Finland	39	7-37 months	Acute gastroenteritis of <7 days duration	5 days	Fermented milk	[8]
Millar <i>et al.</i> 1993	Randomized, double blind; 2 arms: 1. Control: milk feeds 2. Experimental: milk feeds with LGG	Princess Ann Hospital, Southampton, UK	20	Preterm infants	Preterm infants <33 wks	2 weeks	Milk feeds	[9]
Stansbridge <i>et al.</i> 1993	Randomized, double blind; 2 arms: 1. Control: standard milk feeds 2. Experimental: same formula with LGG	Princess Ann Hospital, Southampton, UK	20	Preterm infants	Preterm infants <33 wks	2 weeks	"Standard milk feeds"	[10]
Sepp <i>et al.</i> 1993	Randomized, controlled; 2 arms: 1. Control: breast milk 2. Experimental: breast milk supplemented with LGG	Tampere University Hospital, Finland	25	< 1 month	Term infants	2 weeks treatment	Breast milk	[11]
Buts <i>et al.</i> 1993	Open trial; 1 arm: 1. Experimental: <i>Saccharomyces boulardii</i>	Cliniques St-Luc, University Hospital Brussels	19	Median 8 months	Enterol symptoms for >15 days <i>C. difficile</i> positive stool cultures and positive for cytotoxin B assay	1 month	Lyophilized form	[12]
Saavedra <i>et al.</i> 1994	Double blind, randomized placebo-controlled; 2 arms: 1. Control – Nan2 casein based infant formula 2. Experimental- same formula with added <i>B. lactis</i> Bb12 & <i>S. thermophilus</i>	Baltimore, MD- chronic care facility	55	5-24	Chronically ill	17 months	Nan2, casein based infant formula	[13]
Majamaa <i>et al.</i> 1995	Randomized, double blind; 3 arms: 1. Experimental: LGG 2. Experimental: <i>L. rhamnosus</i> 3. Experimental: <i>S. thermophilus</i> + <i>L. delbrueckii</i> subsp. <i>bulgaricus</i> + <i>L. rhamnosus</i>	Tampere University Hospital, Finland	49	6 to 35 months	Rotavirus-positive with 20 or more watery stools in the 24 hours preceding admission	5 days	Dry powder mixed with 5 ml of water	[14]
Langhendries <i>et al.</i> 1995	Double blind, randomized placebo- controlled; 3 arms: 1. Control: BioNan 2. Experimental: BioNan acidified with <i>S.thermophilus</i> & <i>L. helveticus</i> and supplemented <i>B. lactis</i> Bb12S. 3. Human milk fed (HM)	Rocourt, Belgium	20 formula, 14 HM	0-2	Healthy full term infants	2 months	BioNan, whey dominant formula	[15]

(Table 1) contd....

Author/Year	Design	Location	N	Age at Enrollment	Health Status	Length of Study	Vehicle	Ref.
Kaila <i>et al.</i> 1995	Randomized, double blind; 2 arms: 1. Experimental: Viable LGG 2. Experimental: Heat-killed LGG	Tampere University Hospital, Finland	26	1.3-38.4 months	Acute gastroenteritis of <7 days duration	5 days	5 ml water	[16]
Isolaure <i>et al.</i> 1995	Randomized, double blind, placebo-controlled; 2 arms: 1. Control: capsule of microcrystalline cellulose 2. Experimental: capsule with LGG	Tampere University Hospital, Finland	57	60-150 days	Normal, healthy	5 days	Capsule mixed with 5 ml water	[17]
Raza <i>et al.</i> 1995	Prospective, randomized, double blind, placebo controlled; 2 arms: 1. Control: placebo of microcrystalline cellulose 2. Experimental: LGG freeze-dried prep	Infectious Disease Hospital in Peschawar, Pakistan	38	1-24 months	Acute diarrhea, at least moderate dehydration per WHO	2 days	Mixed with 10 ml of ORS	[18]
Siigur <i>et al.</i> 1996	Randomized; 3 arms: 1. Experimental: antibacterial drug (ABD) 2. Experimental: LGG 3. Experimental: ABD + LGG	Children's Clinic of the University of Tartu, Estonia	33	1-13 years mean age (5.3 years)	Acute diarrhea	10 days	N/A	[19]
Kok <i>et al.</i> 1996	Randomized, double blind, placebo-controlled; 2 arms: 1. Control: unspecified infant formula 2. Experimental – same formula with <i>Bifidobacterium</i> spp. LW420	Netherlands	17	0-3 months	Normal, healthy	12 weeks	Infant formula	[20]
Shornikova <i>et al.</i> 1997	Randomized, double-blind; 2 arms: 1. Control/placebo: nonfat dry milk powder 2. Experimental: <i>L. reuteri</i>	Tampere University Hospital, Finland	40	6-36 months	Acute diarrhea <7 days	Treatment ≤5 days, 1 month follow-up	50-100ml fluid of subject choice	[21]
Guerin-Danan <i>et al.</i> 1998	Randomized; 3 arms: 1. Control: non-fermented gelled milk 2. Experimental: fermented milk with traditional yogurt cultures <i>Streptococcus thermophilus</i> and <i>L. bulgaricus</i> 3. Experimental: fermented milk with same traditional yogurt cultures + <i>L. casei</i>	8 urban day care centers in Val de Marne, France	39	10-18 months	Healthy	1 month	Fermented milk	[22]
Phuapradit <i>et al.</i> 1999	Randomized, control; 3 arms: 1. Control: Nan 2 formula 2. Experimental: Nan2 with <i>B. lactis</i> 3. Experimental: Nan 2 <i>B. lactis</i> + <i>S. thermophilus</i>	Bangkok, Thailand-Pakkred Babies Home	175	6-36 months	Healthy infants and children	8 months	Nan2 milk based follow-up formula	[23]
Urao <i>et al.</i> 1999	Open label trial, 1 arm: 1. Experimental: <i>Enterococcus faecalis</i> at T-110 10 ⁸ CFU/ g, <i>Clostridium butyricum</i> To-A at 10 ⁷ CFU/ g, <i>Bacillus mesenteries</i> To-A at 10 ⁶ CFU/g	Tokyo, Japan	9	1-12 months	Recently undergone surgery, medically stable	2 weeks	N/A	[24]

(Table 1) contd....

Author/Year	Design	Location	N	Age at Enrollment	Health Status	Length of Study	Vehicle	Ref.
Oberhelman <i>et al.</i> 1999	Randomized, double-blind placebo-control; 2 arms: 1. Control/placebo: capsule of cellulose microcrist, the powder base for LGG 2. Experimental: capsule of LGG	San Juan - a shanty town community near Lima, Peru	204	6-24 months	Undernourished (lower quartile weight-for-age)	15 months	1 ounce of liquid cherry gelatin	[25]
Pedone <i>et al.</i> 1999	Randomized, blinded 1. Control: jellied milk (JM) 2. Experimental: standard yogurt (Y) 3. Experimental: fermented milk by yogurt cultures and <i>L. casei</i> (YC)	12 day care centers in Val de Marne, Paris	287	6-36 months	Healthy	6 months (3month treatment, 3 month f/u)	Jellied milk (JM), standard yogurt (Y), or fermented milk by yogurt cultures and <i>L. casei</i> (YC)	[26]
Arvola <i>et al.</i> 1999	Randomized, double-blind, placebo-controlled; 2 arms: 1. Control/placebo: capsule of microcrystalline cellulose 2. Experimental: capsule of LGG	Health Care Center of the City of Tampere or Tampere University Hospital, Finland	119	2 weeks to 12.8 years	Respiratory infections on antimicrobial agents	3 months	Capsule	[27]
Vanderhoof <i>et al.</i> 1999	Randomized, double-blind; 2 arms: 1. Control/placebo: capsule of inulin 2. Experimental: capsule of inulin and LGG	Primary care pediatric practice in Midwest city, USA	188	6 months-10 years	Receiving oral antibiotic therapy	10 days	Capsule	[28]
Isolaure <i>et al.</i> 2000	Randomized, double-blind, placebo-controlled; 3 arms: 1. Control/placebo: extensively hydrolyzed whey formula 2. Experimental: same formula with LGG 3. Experimental: same formula with <i>B. lactis</i> Bb12	Finland	27	Mean age 4.6 months	Early onset atopic eczema	6 months	Extensively hydrolyzed whey formula	[29]
Simakachorn <i>et al.</i> 2000	Randomized, double-blind, placebo control; 2 arms: 1. Control/placebo: sachet 2. Experimental: sachet with <i>L.acidophilus</i> LB	Maharat Nakhon Ratchasima Hospital, Thailand	73	3-24 months	Acute diarrhea, mild-moderate dehydration	4 days	Sachets mixed with 15 ml water	[30]
Guandalini <i>et al.</i> 2000	Randomized, double-blind, placebo control; 2 arms: 1. Control/placebo: ORS 2. Experimental: ORS with LGG	Multicenter in Europe	287	1-36 months	Acute diarrhea	30 days	Sachet added to ORS	[31]
Kalliomaki <i>et al.</i> 2001	Randomized, double blind, placebo-controlled; 2 arms: 1. Control/placebo: capsule of microcrystallized cellulose 2. Experimental: capsule of LGG	Finland	159	0-6 months	Family history of atopic disease	6 months (2 year follow-up)	Capsule	[32]
Boudraa <i>et al.</i> 2001	Randomized, double-blind; 2 arms: 1. Control: infant formula 2. Experimental infant formula with <i>L. bulgaricus</i> plus <i>S. thermophilus</i>	Amilcar Cabral Clinic, Oran, Algeria	112	3-24 months	Acute watery diarrhea, well-nourished	7 days	Infant formula	[33]

(Table 1) contd....

Author/Year	Design	Location	N	Age at Enrollment	Health Status	Length of Study	Vehicle	Ref.
Rautava <i>et al.</i> 2002	Randomized, double-blind, placebo-control; 2 arms: 1. Control/placebo: microcrystalline cellulose 2. Experimental: LGG	Turku, Finland	62 mother infant pairs	Mother age-N/A. Infants-day of life 1	Family history of atopy	4 months (4 weeks prior to birth until infant reached 3 months)	Capsule	[34]
Mastretta <i>et al.</i> 2002	Randomized, double-blind 1. Control/ placebo: capsule of inert oligosaccharides 2. Experimental: capsule of LGG	Department of Pediatrics, University of Turin, Italy	220	1-18 months	Children admitted with common diseases (see comments)	Control: mean 5.1 days, LGG group: 5.33 days plus 4 days post discharge	Capsule dissolved in water	[35]
Kankaanpää <i>et al.</i> 2002	Randomized, double-blind, placebo control; 3 arms: 1. Control/placebo: extensively hydrolyzed formula 2. Experimental: same formula with LGG 3. Experimental: same formula with <i>B. lactis</i> Bb12	Pediatric clinic in Turku, Finland	15	Mean age 5.2 months	Atopic eczema per Hanifin Criteria	Mean = 5.8 months	Extensively hydrolyzed formula	[36]
Dani <i>et al.</i> 2002	Prospective, randomized, double-blind; 2 arms: 1. Control: breast milk or formula 2. Experimental: breast milk or formula with LGG	12 Italian NICU's	555	Preterm infants	Preterm infants <33 wks gestation or <1500g	Mean Duration: Probiotic group 47.3±26.0 days and Placebo group 48.2±24.3 days	Expressed breast milk or preterm infant formula	[37]
Chandra, RK 2002	Prospective, randomized, double-blind; 2 arms: 1. Control: 1 tsp sterile water 2. Experimental: 1 tsp water with <i>L. sporogenes</i>	Rural India	112	Term infants, exact age N/A	Healthy term infants	12 months	1 tsp sterile water	[38]
Moro <i>et al.</i> 2002	Randomized, placebo controlled; 3 arms: 1. Control/placebo: infant formula with maltodextrin as placebo 2. Experimental: 0.4 g/dL oligosaccharide 3. Experimental: 0.8 g/dL oligosaccharide	Macedonio Melloni Maternity Hospital and Mangia-galli Hospital, University of Milan	90	Mean age Placebo: 6.3±2.1 days 0.4 g/dL OS: 6.8 ± 1.9 days 0.8 g/dL OS: 7.3 ± 2.2 days	AGA term infants	28 days	Infant formula	[39]
Boehm <i>et al.</i> 2002	Randomized, controlled; 2 arms: 1. Control: preterm formula 2. Experimental: preterm formula with oligosaccharide (OS) 3. Fortified human milk as reference group	NICU at Hospital Mangia-galli, Milan	42, 30 formula & 12 human milk	<12 days	Preterm infants- max gestation of 32 weeks	28 days	Preterm formula	[40]

(Table 1) contd....

Author/Year	Design	Location	N	Age at Enrollment	Health Status	Length of Study	Vehicle	Ref.
Agarwal <i>et al.</i> 2003	Prospective, randomized; 2 arms: 1. Control: breast milk 2. Experimental: Expressed breast milk with LGG (Culturelle)	All India Institute of Medical Science in New Delhi	71	1-3 days	Preterm infants weight <2000 g	8 days for infants <1500 g and 21 days for infants 1500-1999g	Expressed breast milk	[41]
Costa-Ribeiro <i>et al.</i> 2003	Randomized, double-blind placebo control; 2 arms: 1. Control/placebo: oral rehydration solution (ORS) 2. Experimental: ORS with LGG	Metabolic unit, Dept of Pediatrics, Federal University of Bahia, Brazil	124	1-24 months	Males with acute diarrhea and a minimum of moderate dehydration (per WHO criteria)	Cessation of diarrhea or up to 7 days	ORS	[42]
Schmelzle <i>et al.</i> 2003	Randomized, double-blind; 2 arms: 1. Control: standard infant formula (SF) 2. Experimental: new formula (NF) GOS, FOS, partially hydrolyzed protein, fat 41% of palmitic acid in β position and starch	Four centers in Germany	102	<2 weeks	Healthy term infants	12 weeks	Infant formula	[43]
Kalliomaki <i>et al.</i> 2003	Randomized, double blind, placebo-controlled; 2 arms: 1. Control: capsule of microcrystallized cellulose 2. Experimental- capsule of LGG	Finland	159	0-6 months	Family history of atopic disease	4 year follow-up	Capsule	[44]
Duggan <i>et al.</i> 2003	Randomized, blinded, control (2 consecutive trials); 2 arms: 1. Control: infant cereal 2. Experimental: cereal with oligofructose	Canto Grande- a shanty town community near Lima, Peru	282 & 349	6-12 months	No chronic illness or congenital abnormalities	6 months	Infant cereal (rice or oat) supplemented with or without oligofructose. Second trial added Zn at 1 mg/15g cereal to both formulas	[45]
Bettler <i>et al.</i> 2003	Randomized, double-blind, controlled; 3 arms: 1. Control: growing up milk formula 2. Experimental: growing up milk with <i>B. lactis</i> BI-07 (probiotic group) 3. Experimental: growing up milk with <i>B. lactis</i> BI-07 and fructo-oligosaccharide (synbiotic group)	Multicenter within the United States	169	12-36 months	Healthy	7 weeks	Growing up milk- (formula designed for 12-36 month old children)	[46]
Bettler <i>et al.</i> 2003	Randomized, color-coded, controlled; 3 arms 1. Control: bovine milk- based infant formula 2. Experimental: bovine milk-based infant formula with 1.5g/L FOS 3. Experimental: bovine milk- based infant formula with 3.0g/L FOS	Multicenter within the United States	212	≤ 14 days of life	Healthy term infants	12 weeks	Bovine milk-based infant formula	[47]

(Table 1) contd....

Author/Year	Amount of Prebiotic or Probiotic / Intake per Day	Variables Measured	Outcomes	Adverse Events	Comments	Ref.
Isolaurei <i>et al.</i> 1991	LGG-fermented yogurt: 125 g (1x 10 ¹⁰⁻¹¹ CFU) 2 doses per day; LGG freeze dried: 1x 10 ¹⁰⁻¹¹ CFU/ dose, at 2 doses per day; Placebo yogurt: 125g, 2 doses per day “insignificant amount of lactic acid bacteria”	1. Duration of diarrhea 2. Intestinal permeability 3. Weight gain	1. Compared with the placebo group, LGG groups experienced a significantly (p<0.001) shorter duration of diarrhea 2. No significant between-group differences in intestinal permeability were observed 3. No between group-differences in weight gain	None reported	Breast feeding allowed	[48]
Kaila <i>et al.</i> 1992	LGG-fermented yogurt: 1x 10 ¹⁰⁻¹¹ CFU/ dose, at 2 doses per day Placebo yogurt: <10 ³ of lactic acid bacteria at 2 doses per day	1. Duration of diarrhea 2. Total number of immunoglobulin secreting cells 3. Rotavirus-specific immune response	1. Compared with the placebo group, LGG group experienced a significantly (p<0.001) shorter duration of diarrhea 2. LGG group evidenced a significant increase in immunoglobulin secreting cell numbers 3. Compared to the placebo group, a significantly greater percentage (90% versus 46%) of the LGG group developed an IgA specific antibody-secreting cell response to rotavirus	None reported		[8]
Millar <i>et al.</i> 1993	LGG at 1 x10 ⁸ CFU given twice per day	1. Fecal microbiology: staphylococci, LGG, <i>Enterobacteriaceae</i> , enterocci, and total anaerobes	1. LGG was detected in 9 out of 10 infants in the LGG group 2. No between-group differences were observed for other members of the fecal microflora	None reported	Milk feeds consisted of expressed breast milk, term formula and preterm formula	[9]
Stansbridge <i>et al.</i> 1993	LGG at 1 x10 ⁸ CFU given twice per day	1. Fecal SCFAs and ethanol 2. Urinary 2,3-butanediol 3. Fecal microbiology	1. LGG was detected in 9 out of 10 infants in the LGG group 2. No between-group differences were found in fecal SCFA's or in urinary 2, 3-butanediol 3. Ethanol was detected in more fecal samples from infants fed LGG and at higher concentrations	None reported		[10]
Sepp <i>et al.</i> 1993	LGG 10 ¹⁰⁻¹¹ CFU/g	1. Fecal bacteria composition of meconium 2. Colonisation of <i>Lactobacillus casei</i> GG 3. Microbiota during 1 st week of life	1. Meconium similar in both groups 2. <i>Lactobacillus casei</i> GG present in 67% (10/15) experimental group & 8/10 2 weeks after supplementation 3. Infants 3-4d, 5-7 d, and 1 month old in the experimental group: lactobacilli significantly exceed control. At one month, experimental group more colonized with bifidobacteria than control	None reported	-All breast fed 1 st week of life - No subjects received antibiotics	[11]
Buts <i>et al.</i> 1993	<i>S. boulardii</i> 250 mg twice per day for <1 year, three times for 1-4 years and 4 times for <4 years	1. Symptoms and clinical findings 2. Number of stools, frequency of colic episodes and total duration of colic per day 3. Clearing of cytotoxin and <i>C. difficile</i>	1. 18/19 subjects – symptoms and clinical findings resolved within 1 week of treatment 2. On day 7, number of stools, frequency of colic episodes and total duration of colic per day decreased significantly (p <0.001) 3. Cytotoxin B cleared in 16 subjects by day 15, and in 1 month for 2 subjects. <i>C. difficile</i> gone from stool sample at 1 month for 14 subjects 4. Relapse in 2 subjects, successfully treated with second 15 day treatment	None reported		[12]

(Table 1) contd....

Author/Year	Amount of Prebiotic or Probiotic / Intake per Day	Variables Measured	Outcomes	Adverse Events	Comments	Ref.
Saavedra <i>et al.</i> 1994	<i>B. lactis</i> Bb12: 1.9×10^8 CFU/g formula (35.8×10^8 CFU/100kcal) <i>S. thermophilus</i> : 0.14×10^8 CFU/g formula (2.69×10^8 CFU/100kcal) Mean intake = probiotic group: 701 mL/d Control group: 767 mL/d	1. Occurrence of diarrhea 2. Faecal samples analyzed for rotavirus antigen 3. Feeding tolerance (regurgitation, emesis) 4. Growth – weight and length	1. Significantly fewer cases of acute diarrhea in probiotic group $p=0.035$. Severity of diarrhea was similar amongst 2 groups 2. Rotaviral diarrhea decreased from 19%-7% with probiotics -significantly lower rate of rotaviral shedding 3. No adverse events associated with feeding 4. Adequate growth for all infants	None reported	-Demonstrated tolerance of probiotics in a nutritionally at risk population (immunocompromised due to chronic illness)	[13]
Majamaa <i>et al.</i> 1995	(1) LGG – 5×10^9 CFU/g (2) <i>L. rhamnosus</i> – 5×10^9 CFU/g (3) <i>S. thermophilus</i> (2.66×10^9 CFU/g), <i>L. delbrückii</i> subsp. <i>bulgaricus</i> (1.12×10^8 CFU/g) + <i>L. rhamnosus</i> (2.8×10^7 CFU/g) Probiotic preparations mixed with 5 ml of water and administered twice daily	1. Serum antibodies to rotavirus 2. Total number of immunoglobulin-secreting cells 3. Specific antibody-secreting cells to rotavirus	1. LGG administration shortened duration of diarrhea ($P=0.04$) 2. No differences in total number of immunoglobulin-secreting cells 3. An increase in specific antibody-secreting cells to rotavirus was observed in the LGG group	None reported	No control group	[14]
Langhendries <i>et al.</i> 1995	<i>B. lactis</i> Bb12: 10^6 viable bacteria/g formula Ad libitum feeding Intake not reported	1. Tolerance/acceptability 2. Growth-anthros @ 0,1,2 months 3. Faecal flora composition 4. Stool pH	1. No significant difference in acceptability and tolerance amongst 3 groups 2. Normal growth in all 3 groups 3. Probiotic group had a bifidobacteria count at 1 month similar to HM fed group At 1 month, stool pH significantly lower in control compared to HM but no difference in probiotic group and HM group	None reported		[15]
Kaila <i>et al.</i> 1995	Viable LGG: LGG: 1×10^{10} CFU/ day, twice daily Heat-killed LGG: heat killed equivalent of 1×10^{11} CFU/ day, twice daily	1. Specific rotavirus antibody-secreting cells 2. Total number of immunoglobulin secreting cells 3. Rotavirus IgA and IgG serum antibodies	1. No between-group differences in duration of diarrhea 2. Rotavirus-specific IgA antibody cell response was significantly ($p=0.05$) greater in the LGG supplemented group 3. Serum concentration of rotavirus-specific IgA was greater ($p=0.04$) in viable LGG group at convalescence, but no between-group differences were detected during the acute phase of diarrhea	None reported		[16]
Isolaure <i>et al.</i> 1995	LGG: 5×10^{10} CFU/capsule, at 2 capsules/day	Following administration of oral rotavirus vaccine: 1. Specific rotavirus antibody-secreting cells 2. Total number of immunoglobulin secreting cells 3. Rotavirus IgA, IgM and IgG serum antibodies	1. Compared with the placebo group, the percentage of infants in the LGG group with an increase in rotavirus-specific IgM-secreting cells was significantly greater ($p=0.008$) than in the placebo group 2. Rotavirus-specific IgA seroconversion was significantly ($p=0.05$) greater in the LGG supplemented group 3. No between-group differences in the rotavirus-specific IgM or IgG seroconversion	None reported		[17]

(Table 1) contd....

Author/Year	Amount of Prebiotic or Probiotic / Intake per Day	Variables Measured	Outcomes	Adverse Events	Comments	Ref.
Raza <i>et al.</i> 1995	LGG: 10^{10-11} CFU mixed with ORS every 12 hours	1. Duration and frequency of vomiting and diarrhea 2. Stool samples for pH and reducing substances	1. Frequency of vomiting and diarrhea less in <i>Lactobacillus</i> GG group 2. The % of children with persistent watery diarrhea was significantly less in <i>Lactobacillus</i> GG group ($p < 0.01$) 3. <i>Lactobacillus</i> GG group had more acidic stools at 48 hrs and were less frequently positive for reducing substances than control - not statistically significant	Myoclonic jerks - incidence in both groups (both were malnourished with severe dehydration)	-Children were undernourished per wt for age -Prior antibiotic usage higher in LGG group	[18]
Siigur <i>et al.</i> 1996	LGG: 10^{10-11} CFU/d	1. SCFA at admission and post treatment	1. Increased propionic acid by day 5 in experimental group 2. At 10 days, isocaproic acid was not detected in infants in the LGG group, but was detectable in half of the control infants	1 child in ABD + GG developed allergic skin reaction and emesis		[19]
Kok <i>et al.</i> 1996	<i>Bifidobacterium</i> sp. LW420: 1×10^6 CFU/g formula Not reported	1. PCR detection of <i>Bifidobacterium</i> spp. LW420 in feces	1. <i>Bifidobacterium</i> spp. LW420 detected in experimental group, but not in control group	None reported		[20]
Shornikova <i>et al.</i> 1997	10^{10-11} CFU/d of <i>L. reuteri</i>	1. Rotavirus on admission 2. Duration of diarrhea	1. 75 % subjects had rotavirus (63% in <i>L. reuteri</i> and 86% in placebo). Mean duration of water diarrhea: 1.7 days in <i>L. reuteri</i> and 2.9 days in placebo 2. Mean (SD) of diarrhea until treatment for all subjects was 3.0 (1.7) day	None reported other than expected diarrhea and vomiting		[21]
Guerin-Danan <i>et al.</i> 1998	Yogurt group - <i>L. bulgaricus</i> at 3.0×10^6 CFU/g & <i>S. thermophilus</i> at 1.7×10^9 CFU/g Yogurt + <i>L. casei</i> group: <i>L. bulgaricus</i> at 3.0×10^6 CFU/g, <i>S. thermophilus</i> at 1.7×10^9 CFU/g, <i>L. casei</i> at 9.1×10^9 CFU/g 125 g/ day of respective product	1. Fecal microbiology 2. Fecal pH, moisture, bacterial metabolites 3. Fecal bacterial enzymes	1. No significant differences or changes in total anaerobes, bifidobacteria, bacteroides, and enterobacteria 2. No significant difference in stool pH, fecal water content, fecal β -galactosidase or α -glucosidase activity 3. In yogurt group, a significant increase in fecal enterococci and a significant decrease in fecal branched-chain and long-chain fatty acids were observed 4. In yogurt + <i>L. casei</i> group, the percentage of children with $>6 \log_{10}$ CFU/g feces of lactobacilli increased	None reported	- Breast feeding allowed ad lib	[22]
Phuapradit <i>et al.</i> 1999	<i>B. lactis</i> Bb12: 1×10^8 CFU/g Not reported	1. Incidence of acute diarrhea 2. Salivary IgA, salivary protein, salivary IgA anti-rotavirus	1. 81 episodes of diarrhea-rotavirus accounted for 3.7% of cases. More episodes in experimental groups- shigella responsible for 1/5 of episodes 2. Significant decrease in salivary IgA in the Bb12 + <i>S. thermophilus</i> group, significant increase in salivary IgA anti-rotavirus in control ($p=0.01$), protein levels the same at study end	None reported		[23]

(Table 1) contd....

Author/Year	Amount of Prebiotic or Probiotic / Intake per Day	Variables Measured	Outcomes	Adverse Events	Comments	Ref.
Urao <i>et al.</i> 1999	<i>E. faecalis</i> at 10^8 CFU/g, <i>C. butyricum</i> at 10^7 CFU/g, <i>B. mesenteries</i> at 10^6 CFU/g Intake not reported Two grams of probiotic preparation per day	1. Fecal anaerobes, aerobes, bifidobacteria, <i>E. coli</i> , streptococci, <i>Pseudomonas</i> , klebsiella and clostridia 2. Serum endotoxin and C-reactive protein, 3. White blood cell count	1. Following probiotic administration, fecal anaerobes and bifidobacteria increased significantly ($p=0.05$), whereas streptococci decreased significantly ($p=0.05$) 2. Serum endotoxin concentrations decreased significantly ($p<0.03$) after probiotic administration	None reported	- Of the 9 infants, 5 had biliary atresia, 2 had omphalocele and 1 had Hirschsprung's disease and 1 with high perforated anus	[24]
Oberhelman <i>et al.</i> 1999	1 capsule of placebo or 3.3×10^{10} CFU of LGG for 6 days of the week	1. Number of episodes of diarrhea 2. Duration of diarrhea 3. Fecal presence of LGG 4. Microbiological analysis of stool from participants with diarrhea	1. LGG group experienced significantly fewer episodes of diarrhea 2. No significant difference in duration of diarrhea between groups 3. Adenoviral diarrhea was more common in placebo group	None reported	- Decrease in diarrhea incidence in LGG group was largely limited to non-breastfed children	[25]
Pedone <i>et al.</i> 1999	JM: none Y: traditional yogurt cultures <i>L. bulgaricus</i> and <i>S. thermophilus</i> YC: traditional yogurt cultures <i>L. bulgaricus</i> and <i>S. thermophilus</i> and <i>L. casei</i> (strain DN-114 001) Y and YC-at least 10^7 yogurt bacteria CFU/ml 125g serving for 6-18 month old or 250g for >18 months Y and YC: at least 10^7 yogurt bacteria CFU/ml *Actual intake not reported	1. Incidence of diarrhea 2. Magnitude of diarrhea	1. 26% total children had one or more episodes of diarrhea - no significant difference between groups. 84% only one episode of diarrhea, 14.5% two episodes. Total of 82 episodes over 6 months, 1.2 per child 2. YC significantly lower severity of diarrhea compared to JM, ($p=0.009$). Y and YC both significantly shorter duration of one episodes than JM ($p=0.002$). YC returned to normal stools faster than Y, JM ($p=.0055$)	None reported	- 10% withdrew (no difference between groups) - JM, made from texturisers - ITT not included in analysis	[26]
Arvola <i>et al.</i> 1999	2×10^8 CFU of LGG twice daily	1. Incidence of diarrhea during 2 weeks from start of antibiotics 2. Activities of fecal urease, β -glucuronidase and β -glucosidase	1. Incidence was 5% in LGG group and 16% in placebo. Treatment effect was 11% 2. Severity of diarrhea comparable between groups 3. Activities of fecal urease, β -glucuronidase but not β -glucosidase changed significantly in both groups with start of antibiotics. Levels normalized by 3 weeks	None reported	- Subjects had not received antibiotics for 3 months prior	[27]
Vanderhoof <i>et al.</i> 1999	Children <12 kg: 1 capsule (10 billion CFU LGG) per day >12 kg: 2 capsules per day	1. Incidence and duration of diarrhea 2. Stool consistency	1. 26% of placebo had diarrhea vs 8% in LGG group. Mean duration of diarrhea was 4.70 in LGG and 5.88 in placebo ($p=0.05$) 2. LGG significantly reduced stool frequency and increased consistency compared to control at day 10 ($p<0.02$)	None reported		[28]

(Table 1) contd....

Author/Year	Amount of Prebiotic or Probiotic / Intake per Day	Variables Measured	Outcomes	Adverse Events	Comments	Ref.
Isolaurei <i>et al.</i> 2000	LGG: 3x 10 ⁸ CFU/g formula Bb12: 1x10 ⁸ CFU/g formula At 2 months- 1) LGG group: 70-82 ml/kg/d or 56% of total kcal 2) <i>B. lactis</i> Bb12 group: 63-88 mL/kg/d or 52% of total kcal At 6 months- Decreased to 56-65% of total kcal *mean intake of probiotics 3x10 ⁸ CFU/d	1. Growth/nutrition 2. Extent and severity of eczema 3. Concentrations of circulating cytokines and chemokines, and soluble cell surface adhesion molecules in serum and methyl-histamine and eosiphilic protein X in urine	1. Normal growth as per length for age, labs within normal limits, comparable formula intake amongst groups 2. Atopic eczema improved per SCORAD score at 2 months in probiotic groups as compared to control (p=0.002). At 6 months the score was similar in all 3 groups 3. CD4 in serum decreased in probiotic groups, and a significant decrease in protein X in urine (Bb 12 group p=0.01, LGG group p=0.04)	None reported		[29]
Simakachorn <i>et al.</i> 2000	2 x 10 ¹⁰ bacteria/day in sachets containing 10 billion <i>L. acidophilus</i> LB and 160 mg fermented lyophilized culture medium	1. Duration of diarrhea 2. Rotavirus – pre/post treatment 3. Stool consistency	1. Significantly reduced duration of diarrhea in <i>L. acidophilus</i> LB group (marked decrease in children with no antibiotic therapy) 2. Rotavirus-found in approximately 50% of each group and after treatment rotavirus-positive with watery stools was significantly lower in <i>L. acidophilus</i> LB group	None reported	- Children started on ORS initially then formula or breast milk and rice gruel	[30]
Guandalini <i>et al.</i> 2000	At least 10 ¹⁰ CFU/250mL	1. Duration of diarrhea 2. Number of watery stools 3. Weight gain 4. Duration of hospital stay	1. Duration of diarrhea significantly less in <i>Lactobacillus</i> GG vs control (p=0.03). In rotavirus positive children, shorter duration than control (p<0.008) 2. Watery stools were significantly reduced in rotavirus positive children in the <i>Lactobacillus</i> GG group 3. <i>Lactobacillus</i> GG group had a better average weight gain- not significant 4. Hospital stay significantly shorter in <i>Lactobacillus</i> GG group	None reported		[31]
Kaliomaki <i>et al.</i> 2001	LGG: 1x 10 ¹⁰ CFU/ capsule 2 capsules per day	1. Atopic eczema presence 2. Serum total IgE 3. Serum specific IgE 4. Skin prick test	1. LGG supplementation significantly (p=0.008) reduced the frequency of atopic disease to half of that of the placebo-group 2. No between-group differences in total IgE 3. No between-group differences in serum IgE 4. No between-group differences in skin prick tests	None reported		[32]
Boudraa <i>et al.</i> 2001	10 ⁸ CFU/g <i>L. bulgaricus</i> and 10 ⁸ CFU/g <i>S. thermophilus</i> - Daily dose not available	1. Presence of reducing sugars in stool 2. Cessation of diarrhea and appropriate weight gain within 7 days 3. Duration of diarrhea	1. Similar between groups 2. 82 % control formula and 84% in probiotic group. Failure rate of 3.6% in both groups 3. Duration and # of stools significantly less in probiotic group	One subject from each treatment had weight loss and persistent diarrhea	- formulas same texture and flavor, but mothers could tell difference - children also consumed vegetables, fat, cereal and breast milk - drop-out rate similar amongst treatment groups	[33]

(Table 1) contd....

Author/Year	Amount of Prebiotic or Probiotic / Intake per Day	Variables Measured	Outcomes	Adverse Events	Comments	Ref.
Rautava <i>et al.</i> 2002	LGG: 2×10^{10} CFU/d	1. Serum total IgE measured from cord blood 2. TGF- β_1 and TGF- β_2 measured from breast milk at 3 months 3. Risk of atopic eczema	1. No significant difference in cord blood IgE 2. TGF- β_2 higher in probiotics group 3. Risk significantly reduced in probiotics group compared to placebo 4. Maternal atopy was a risk factor for infant	No adverse events	- 159 women randomized 4 weeks before birth. Subjects were withdrawn in unable to breast feed and take supplement until child reached 3 months	[34]
Mastretta <i>et al.</i> 2002	2 capsules of 10^{10} CFU LGG upon admission and then 1 capsule daily	1. Total incidence of nosocomial rotavirus 2. Attack rate of rotavirus in probiotics and placebo 3. Breast feeding rate 4. Attack rate of rotavirus in breast fed and non-breast fed	1. Total incidence 27.7% (61 of 220) 2. Probiotics group 25.4% (29 of 114) and placebo 30.2% (32 of 106). Difference between groups not significant	No unexpected adverse events	- Children with enteritis, re-admission within 72hrs, gastroenteritis or history of immunodeficiency were excluded	[35]
Kankaanpää <i>et al.</i> 2002	3×10^8 CFU/g LGG & 1×10^9 CFU/g Bifidobacterium Bb12 Mean formula intake: Placebo group -75mL/kg LGG group- 76mL/kg B. lactis Bb12 group- 73mL/kg * not able to determine probiotic intake	Analysis of fatty acids	1. No difference in baseline neutral lipid proportions except probiotics groups had higher eicosapentaenoic acid 2. Plasma neutral lipids, α -linolenic acid reduced with probiotics 3. Phospholipids - Bb12 significantly increased the proportion of α -linolenic acid, LGG did not	None reported	- All infants previously breast fed	[36]
Dani <i>et al.</i> 2002	6×10^9 CFU /d LGG	1. NEC incidence 2. UTI incidence 3. Sepsis incidence	1. 1.4% NEC in probiotics group, 2.8% in placebo- not significant 2. 34% UTI in probiotics group, 5.2% in placebo- not significant 3. 4.7% sepsis in probiotics group, 4.1% in placebo- not significant	No unexpected adverse events	-Probiotics or placebo supplementation started with oral feeds -NEC diagnosed by Bell's criteria, $\leq$ stage 2 -Antibiotic regimen similar between groups	[37]
Chandra <i>et al.</i> 2002	100 million/d <i>L. sporogenes</i>	1. # of rotavirus episodes (ELISA) 2. Duration of rotavirus episodes 3. # days ill 4. # infants with dehydration 5. Body weight at one year	1. Lactobacillus group had fewer episodes ($P < 0.02$) 2. Duration was shorter in Lactobacillus group ($P < 0.05$) 3. Lactobacillus group fewer days ill ($P < 0.01$) 4. No difference in dehydration at first examination 5. Body weight slightly higher in Lactobacillus group-not significant	None reported	-All infants breast fed 1 st week of life - Other milks (buffalo, goat, cow) were introduced - No difference in breast feeding, formula feeding or other foods consumed between groups	[38]

(Table 1) contd....

Author/Year	Amount of Prebiotic or Probiotic / Intake per Day	Variables Measured	Outcomes	Adverse Events	Comments	Ref.
Moro <i>et al.</i> 2002	0.4 g/dL or 0.8 g/dL oligosaccharide (90% GOS, 10% FOS) Mean feeding volume (mg/kg/d): Placebo: 162±58 0.4 g/dL OS: 163 ± 64 0.8 g/dL OS: 173 ± 55	On Day 1 & Day 28: 1. Fecal species, CFUs, and pH were measured 2. Stool characteristics, growth and side effects recorded	1. Bifidobacteria and lactobacilli similar among 3 formula groups at Day 1 2. Bifidobacteria significantly increased in OS formula groups compared to placebo at Day 28 and the effect was dose dependent (0.4g/dL vs 0.8g/dL p<0.01) 3. Lactobacilli significantly increased in OS groups compared to placebo - no significant difference between OS formula groups 4. OS had no effect on other fecal species 5. pH was significantly changed: increased in placebo group, no significant change in the 0.4 g/dL group and decreased in the 0.8g/dL 6. Stool frequency increased in 0.8 g/dL group and stools became softer. Stools became harder in placebo group 7. No difference among groups in side effects or growth	Data not shown		[39]
Boehm <i>et al.</i> 2002	1g OS/100ml (90% GOS, 10% FOS) Mean feeding volume: Control formula: 175.6 ml/kg/d OS formula: 171.5 ml/kg/d Human milk: 186.7 ml/kg/d 0.3 g FOS/d and 2.7 g GOS/d	Day 1, 7, 14, 28 evaluation of 1. Faecal flora 2. Stool characteristics 3. Growth 4. Side effects	1. Bifidobacteria in OS group reached upper range of reference group. Difference between formula groups (P=0.0008) 2. Stool frequency after 28days significantly lower in control than OS (P=0.0079). Stools became harder in control group - fairly stable in OS and reference group 3. No difference in weight or length gain 4. No difference in side effects	None reported	All infants initially fed pasteurized mother's milk and supplemented with a human milk fortifier when tolerating 80ml/kg/d	[40]
Argawal <i>et al.</i> 2003	Expressed breast milk supplemented twice per day with 10 billion lyophilized cells per unit LGG	1. Stool samples pre- and post-treatment for quantitative aerobic and anaerobic cultures	1. LGG colonization in 17/47 of all treated infants vs 0/24 in control infants (P= 0.005) 2. <1500 g infants: LGG colonization on day 14 in 4% (1/24) and on day 21 in 21% (5/24) 3. 1500 –1999g infants: LGG colonization by day 4 in 17% (4/23) and on day 8 in 47% (11/23). No significant difference in gram (+) or gram (-) species. 10/11 colonized and 10/12 non-colonized on day 8 did not receive antibiotics	None reported		[41]
Costa-Riberio <i>et al.</i> 2003	10 billion CFU/ d LGG Intake: ORS 100ml/kg x first 6hrs and then ORS prn to replace volume of lost stool	1. Duration of diarrhea 2. Stool output 3. Requiring IV 4. Vomiting within 24 hrs of randomization 5. Hyponatremia 24 hrs of randomization 6. Rotavirus	1. Mean duration (hrs): LGG 38.27 hrs and Control 39.09 hrs 2. No significant reduction in diarrhea between groups 3. No difference in ORS, breast feeding or IV 4./5. No vomiting or hyponatremia reported 6. 50% Rotavirus in LGG and 48% in Control. No significant difference in diarrhea or stool output	None reported	- Breast feeding allowed ad lib	[42]

(Table 1) contd....

Author/Year	Amount of Prebiotic or Probiotic / Intake per Day	Variables Measured	Outcomes	Adverse Events	Comments	Ref.
Schmelzle <i>et al.</i> 2003	0.8 g/100ml oligosaccharide (90% GOS, 10% FOS) Mean intake: NF at 6 wks: 135ml/kg/d, estimated oligo-saccharide 5.5 g/d or 1.08 g/kg NF at 12 wks: 111 ml/kg/d or estimated oligosaccharide 5.7g or 0.89g/kg	1. Gains in weight, length, head circumference (HC) 2. Skin fold thickness 3. Protein status 4. Fecal bacteriology 5. Formula intake 6. Formula acceptance	1. NF girls gained more weight at 6 wks ($P<0.05$) and HC at 12 wks ($P<0.05$). No difference in length or weight gain at 12 weeks 2. NF boys gained more total skinfold thickness ($P<0.05$) 3. At 6wks - total protein, albumin, urea similar between groups. NF lower prealbumin ($P<0.002$); lower tyrosine ($P<0.05$); higher threonine ($P=0.001$), isoleucine ($P<0.05$) and lysine ($P=0.007$) mean threonine above normal for breast-fed infants 4. NF higher portion of bifidobacteria and softer stools 5. NF consumed less formula and kcal/kg 6. Formula well tolerated >90% of completed infants & mothers report no feeding problems	About 1/3 mothers reported possetting (no difference between formulas)	- NF is 70kcal and 1.7g protein /100ml vs SF is 67 kcal and 1.5 g protein/100ml - 34% drop-out rate, mainly due to feeding problems. Not statistically different between groups	[43]
Kaliomaki <i>et al.</i> 2003	LGG: 1×10^{10} CFU/capsule 2 capsules per day	1. Atopic eczema presence 2. Bronchial inflammation via exhaled nitric oxide 4. Skin prick test	1. LGG supplementation significantly reduced the relative risk of atopic eczema to 0.57 frequency of atopic disease to half of that of the placebo-group 2. No significant between-group differences in seasonal allergic rhinitis or asthma incidence 3. No between-group differences in skin prick reactivity tests	None reported		[44]
Duggan <i>et al.</i> 2003	18.4 ± 10.4 g/d cereal or 0.67 g oligofructose/d or 0.11g/kg/d	1. Prevalence of diarrhea 2. # of episodes of diarrhea 3. Incidence of severe diarrhea 4. Incidence of persistent diarrhea 5. Nutritional status 6. Postimmunization titer of antibody to Heamophilus influenza type B (Hib)	No significant difference in prevalence, # of episodes, severity of diarrhea, gain in height, clinic visits or response to Hib between cereal types	None reported	- Breast feeding allowed	[45]
Bettler <i>et al.</i> 2003	1×10^8 <i>B. lactis</i> CFU /g and 1.5 g FOS/L Mean intake of formula: 233 mL/d	1. Presence of <i>B. lactis</i> in stool samples at baseline, weeks 1, 4, 6 2. Change in fecal bacteria in stool samples at baseline, weeks 1, 4, 6 3. Assess and compare acceptability, tolerance of formula and adverse events	1. All samples negative for <i>B. lactis</i> at baseline. Week 1: 39% positive in probiotic group, 38% in synbiotic group. Week 4: 41% positive in probiotic group and 50% in synbiotic group. Week 6 (2 weeks post study feeding) 1% positive in probiotic, 0% in synbiotic. 2. Significant increase in bifidobacteria compared to control at week 1(per protocol analysis) but no difference at week 4 of 6 3. No statistical difference in acceptability, tolerance or adverse events among formula groups. Less diarrhea in synbiotic group but not statistically significant	13% of children reported an adverse event related to formula - no difference among study groups	No serious adverse events related to the formula	[46]

(Table 1) contd....

Author/Year	Amount of Prebiotic or Probiotic / Intake per Day	Variables Measured	Outcomes	Adverse Events	Comments	Ref.
Bettler <i>et al.</i> 2003	g/L FOS and 3.0 g/L FOS Feeding ad libitum	1. Evaluate indices of growth: weight, length and head circumference 2. Evaluate safety – adverse events, serum lab markers, and tolerance	1. All three formula effective at promoting growth per CDC reference ranges using percentiles and Z scores 2. All three formulas considered safer per growth data, adverse events profiles, lab data. The 3.0g/L had fewer adverse events than control and less constipation (p=0.0333 in the intent-to-treat)	55% of overall infants had formula-related adverse events	No serious adverse events related to the formula. Most common events were constipation and flatulence. Reported adverse events are common events in this age group	[47]

15, 19, 20, 22, 24, 41]. These outcomes include the detection of specific probiotic strains, as well as the examination of key groups of the gastrointestinal microflora: bifidobacteria, lactobacilli, streptococci, total anaerobes, clostridia, *Enterobacteriaceae* family members, and others. It is important to note that most of these observations relating to the gastrointestinal microflora have been inferred from the microbiological analyses of feces. Several studies have involved examination for the presence of the LGG strain specifically, using a phenotypic analysis, in the feces of infants after feeding. Millar *et al.* [9] and Stainsbridge *et al.* [10] found that 90% of infants fed LGG for 2 weeks evidenced the same microbe in feces, but simultaneous alterations in other members of the flora were not observed. Similarly, Sepp *et al.* [11] observed the presence of LGG in newborns fed the microbe at 10^{10} colony forming units (CFU) per day for 2 weeks. A concurrent increase in fecal lactobacilli and coliform counts was observed at between 3 to 7 days in the LGG group, but at one month the fecal counts of coliforms, bacteroides and lactobacilli were similar for infants in the control and treatment groups. Kok *et al.* [20] used a specific polymerase chain reaction (PCR)-based method to detect the presence of a strain of bifidobacteria, *Bifidobacterium* sp. LW420, in feces of infants fed a formula containing the same microbe. The ingested bifidobacteria were detected in the feces of all infants that were fed the strain, while not in the feces of infants fed the control formula.

In a study with young children, aged 12 to 36 months [46], subjects were randomized to receive formula supplemented with *B. lactis* BI-07 (probiotic group), formula supplemented with *B. lactis* BI-07 and FOS (synbiotic group), or control formula. Stool samples were collected at baseline and at 1, 4, and 6 weeks (2 weeks post-study feeding). The presence of fecal *B. lactis*, using a PCR-based detection method, was monitored, as were changes in key groups of fecal bacteria. Whereas samples were negative for *B. lactis* at baseline, the percentage of infants in the treatment groups with detectable *B. lactis* signal had risen to 39% and 38% in the probiotic group and the synbiotic group, respectively, after one week of feeding. After 4 weeks 41% of subjects in the probiotic group and 50% in the synbiotic group were positive for *B. lactis*. Although the incidence of

diarrhea was lower in the synbiotic group than in the control group, frequency of diarrhea was not great enough to allow statistical conclusions to be drawn.

ACUTE DIARRHEA

Acute diarrhea is a sudden change in stool fluid output that increases the number of bowel movements from 4 to 20 per day [50]. Diarrhea contributes significantly to infant morbidity and mortality, with the World Health Organization estimating that 3 million deaths occur per year as a result of diarrhea [51]. In developing countries, children younger than 5 years of age have an average of 3 episodes of diarrhea per year, compared with 1 to 2 episodes per year in the United States (US) [52]. There are many potential causes of acute diarrhea: infection, drug therapy, food allergies, gastrointestinal disorders, and malnutrition. Acute diarrhea is most commonly caused by infection, with rotavirus accounting for the majority of viral cases. Bacterial infections are more apparent in the early months of infancy, whereas from 6 months to 2 years of age, rotavirus accounts for 25% to 40% of acute diarrheal cases in developed countries [50]. Probiotics have been studied for the management of various types of diarrheal disease, including viral diarrhea, bacterial diarrhea, antibiotic-associated diarrhea, and *Clostridium difficile* diarrhea.

Several investigators have evaluated the impact of probiotics, especially lactobacilli, on recovery from acute diarrhea in hospitalized children. Boudraa *et al.* [33] randomized infants, admitted to the hospital with acute watery diarrhea, to a control infant formula or formula supplemented with *Lactobacillus bulgaricus* and *S. thermophilus*, each at a concentration of 10^8 CFU/g. These investigators observed that the duration was shorter, and the number of stools was significantly lower, in the probiotic group. Isolauri *et al.* [7] studied the effect of LGG on the recovery of acute diarrhea. Infants and children aged 4 to 45 months with acute diarrhea (82% rotavirus) were fed LGG-fermented milk product, LGG freeze-dried powder, or placebo following the administration of oral rehydration therapy. The LGG groups experienced significantly shorter duration of diarrhea, significantly less watery diarrhea, and

no difference in intestinal permeability. The mean duration of diarrhea was reduced by one day in the LGG groups. Raza *et al.* [18] fed the LGG strain mixed in ORS to undernourished children with acute diarrhea. The study took place in a province in Pakistan where diarrhea is endemic and frequently a result of bacterial pathogens. After 48 hours of LGG supplementation, the frequency of diarrhea and emesis was decreased compared to control, and children admitted with non-bloody diarrhea experienced significantly less persistent watery diarrhea. Shornikova *et al.* [21] reported that children hospitalized for acute diarrhea (75% rotavirus) had significantly less watery diarrhea and vomiting on the second day of treatment with *Lactobacillus reuteri* supplement compared with placebo. Simakachorn *et al.* [30] supplemented oral rehydration therapy with heat-killed *Lactobacillus acidophilus* LB in infants and young children aged 3 to 24 months with acute diarrhea (50% rotavirus). Mean duration of diarrhea in the *L. acidophilus* LB group was 43.4 hours, compared with 57 hours in the control group, and subjects positive for rotavirus endured significantly less watery stool after 24 hours of *L. acidophilus* LB treatment. A multicenter trial in Europe involved 140 infants and young children aged 1 to 36 months with acute diarrhea [31]. LGG (10^{10} CFU/250 ml of ORS) or placebo was added to the ORS. The length of stay in the hospital was significantly shorter in groups who received LGG, and the duration of diarrhea was significantly decreased, particularly in the rotavirus-positive children. Diarrhea persisted longer than 7 days in more children in the placebo group. In several other studies, focused specifically on rotaviral diarrhea, investigators have found probiotics to be effective in shortening the duration of the disease. [7, 8, 14, 53]. However, in a 2003 study involving males 1 to 24 months of age with acute diarrhea, no reduction in diarrhea was observed with supplemented LGG at 10^{10} CFU per day in comparison with control subjects [42].

Probiotics may also be useful in the prevention of diarrhea. In a study of ambulatory, undernourished infants and young children in Peru, Oberman *et al.* [25] observed that infants and young children fed 3.3×10^{10} CFU per day experienced significantly fewer episodes of diarrhea than did their control counterparts. Pedrone *et al.* [26] conducted a 6-month study in daycare centers with children consuming *Lactobacillus casei*-fermented milk, standard yogurt, or control milk. The severity of diarrhea decreased significantly with *L. casei* fermented milk compared with control, but no difference in incidence was observed. Szajewska *et al.* [54] found that prophylactic LGG, compared with placebo, significantly reduced the risk of nosocomial diarrhea, and noted that the reduction was greatest for nosocomial rotavirus gastroenteritis. In a study by Saavedra *et al.* [13], infants were fed a formula supplemented with bifidobacteria (10^{10} CFU/d) and *S. thermophilus* (10^9 CFU/d) for a period of 17 months and monitored for the occurrence of diarrhea and rotavirus antigen. The incidence of diarrhea and of rotaviral shedding was significantly lower in the group fed the probiotic-supplemented formula than in those fed control formula. In a study by Chandra [38], infants were fed supplemental *Lactobacillus sporogenes* at a concentration of 10^5 CFU/d and were monitored for episodes and duration of rotaviral diarrhea and number of days of illness. The group

of infants receiving *L. sporogenes* experienced fewer episodes of diarrhea, with a shorter duration of diarrhea and fewer days of illness.

It is important to note that Sanders *et al.* [55] recently pointed out that microbial preparations described as *L. sporogenes* may actually be mislabeled and, in fact, contain *Bacillus coagulans*.

ANTIBIOTIC-ASSOCIATED DIARRHEA

Antibiotics are routinely prescribed for infants and children, but a serious and common side effect of antibiotic therapy is diarrhea. Antibiotic-associated diarrhea (AAD) is reported to develop in 5% to 25% of cases, with the greater amount attributed to broad-spectrum antibiotic therapy [56]. AAD can be separated into 2 main categories: *C. difficile* disease and non-*C. difficile*. Antibiotics can cause disturbances in the gastrointestinal flora, often resulting in an overgrowth of pathogenic bacteria, such as *C. difficile*. Also, by virtue of their impact on the flora, antibiotics can affect the metabolism of carbohydrates and cause osmotic diarrhea.

The co-administration of antibiotics and LGG has been shown to reduce the incidence of non-*C. difficile* AAD [27, 28]. Treatment with LGG reduced the incidence of AAD in children aged 2 weeks to 12.8 years who were being treated for respiratory infections [28], with the incidence of loose stools at 42% in the control group and 17% in the group consuming LGG. Arvola *et al.* [27] also administered LGG to children receiving antibiotics for respiratory infection and observed that infants receiving the lactobacilli experienced a one third reduction in the incidence of diarrhea. Buts *et al.* [12] conducted an open-label, uncontrolled trial with *Saccharomyces boulardii* (number of organisms not defined) in children with persistent *C. difficile* diarrhea, and observed that in 18 of the 19 children in the study, symptoms resolved within one week of probiotic therapy. Cytotoxin B was cleared from stool samples in 16 of 19 subjects at 15 days and from the feces of all of the subjects at one month. Likewise, after one month *C. difficile* was not detected in stool samples obtained from 14 of the 19 infants.

ATOPIC DISEASE

The prevalence and severity of atopic diseases in the industrialized world are increasing [57, 58]. While there is a clear genetic component in the development of atopy, there are circumstantial indications that this increase may be related to a reduced exposure to environmental microbes [59]. The ability of environmental antigens to avert the onset of atopy derives from the identification of two functionally different subsets of T-helper lymphocytes, T-helper 2 (Th2) and T-helper 1 (Th1) lymphocytes. Atopic disease results from an inappropriate, and primarily Th2-type, immune response to allergens and a switch to immunoglobulin E (IgE) production. Alternatively, Th1 lymphocytes inhibit IgE synthesis. After birth, the infant's immature immune system is exposed to an array of allergens, some of which may facilitate the development of the Th2 response. However, it is speculated that this harmful sensitization is less likely to occur when there is simultaneously an adequate microbial exposure, which can direct the immune response away from the Th2 response and toward the Th1 cytokine phenotype.

In support of this hypothesis are several studies involving the exposure of infants with a high risk for atopic disease development to certain probiotic bacteria that have resulted in dramatic reductions in the occurrences of atopic eczema. In a study by Isolauri *et al.* [29], infants (mean age 4.6 months) with evidence of atopic eczema were randomly assigned to one of 3 treatment groups: extensively hydrolyzed whey formula, the same formula with LGG, or the same formula with *B. lactis* Bb12. After 2 months, the extent and severity of atopic eczema were reduced significantly in infants receiving the LGG and *B. lactis* Bb12 strains, whereas at 6 months (at the conclusion of the treatment), no between-group differences in atopic eczema existed. Kalliomaki *et al.* [32] conducted a randomized, double-blind, placebo-controlled trial in 159 infants with a family history of atopic disease. Subjects were randomly assigned to either the control or treatment group, where pregnant mothers received 2 capsules of placebo or 10^{10} CFU of LGG for the final 2 to 4 weeks of pregnancy. After delivery, the breastfeeding mothers either continued to consume the capsules or provided the capsule contents directly to the infant. The infants were examined at 6 months and again at 2 years of age for the presence of atopic eczema, for serum total and specific IgE, and for skin prick test responses to various antigens. While no differences were noted in IgE concentrations or in skin prick test responses, the incidence of atopic eczema in the LGG group at 2 years of age was half that of the placebo group. Recently, a 4-year follow-up on the same cohort was published [44], demonstrating that the preventive effect of perinatal LGG administration on atopic eczema extends to 4 years of life. Observations in this 4-year follow-up study were similar to those made at 2 years; no differences were noted in skin prick test responses, but the incidence of atopic eczema in the LGG group was 57% that of the placebo group.

PREMATURE INFANTS AND PROBIOTICS

The gastrointestinal flora of preterm infants in neonatal intensive care units (NICU) differs from that of term infants [60, 61], which may be attributable, in part, to the use of parenteral nutrition and antibiotic therapy for extended periods. In addition, bifidobacteria colonization can be delayed in this population [61]. The NICU environment predisposes infants to nosocomial infections, and probiotics may help reduce infection by decreasing the overgrowth of pathogenic bacteria.

Studies in preterm infants are limited, and have focused on the tracking of ingested probiotic bacteria [9, 10, 41] and prevention of infection [37]. Twenty preterm infants (median birthweights <1,500 g) were randomized to receive milk feeds with or without LGG cells for 2 weeks to evaluate colonization and alterations in fecal bacteria. LGG-supplemented milk feeds were well tolerated, and the microbe was detected in 9 of the 10 infants in the experimental group; however, no between-group differences were observed for other fecal microbes [9]. Agarwal *et al.* [41] observed the effects of LGG mixed with expressed breast milk on the neonatal microflora. Length of treatment depended upon birthweight; infants weighing <1,500 g received probiotics or control for 21 days, and those infants weighing 1,500 g to 1,999 g were treated for 8 days. LGG was detected in the

feces of 17 of 47 infants in the probiotics group, while remaining undetectable in the control group. No significant differences in bacterial counts existed between control and LGG groups. LGG was detected at low frequencies in infants weighing <1,500 g and the frequency of detection of LGG increased in heavier infants. Stansbridge *et al.* [10] enrolled 20 preterm infants and attempted to assess changes in enteric carbohydrate fermentation by measuring fecal short-chain fatty acids (SCFAs). LGG was detected in the feces of 9 out of 10 infants in the experimental group, but no between-group differences were observed in SCFAs or in urinary 2,3-butanediol levels.

Necrotizing enterocolitis (NEC) is a disease occurring primarily in preterm infants. It is characterized by focal or diffuse ulceration and necrosis of the small bowel and colon [62]. The mortality rate of NEC ranges from 20% to 30% [63]. The etiology of NEC is not clearly understood and appears to be multifactorial, with significant risk factors including prematurity, formula feeding, aberrant bacterial colonization, and intestinal hypoxia [64].

Probiotics may benefit the immature gut by preventing the overgrowth of pathogens, such as members of the *Enterobacteriaceae* family and coagulase-negative staphylococci, which are common pathogens in NICU infections [65]. Infants from 12 Italian NICUs were investigated to assess the impact of LGG on the incidence of urinary tract infections (UTIs), bacterial sepsis, and NEC. At the initiation of oral feeds, 585 preterm infants were randomized to receive either LGG supplementation or placebo [37]. Although the probiotic-treated group experienced fewer UTIs, less NEC, and more bacterial sepsis than the control group, the changes were not significant.

PREBIOTICS

Prebiotics are added to infant formula primarily to stimulate the growth of bifidobacteria and lactobacilli and, therefore, pattern the microflora of human-milk fed infants. Human milk is a rich source of a diverse array of oligosaccharides (OS), which can serve as substrates for the developing microflora. Human milk OS are resistant to enzymatic digestion in the proximal regions of the digestive tract and, as a result, are available to act as competitive ligands for the interaction of pathogens with the gut epithelium [66]. Unlike human milk, cow's milk formula contains only trace amounts of OS, hence studies have been conducted to evaluate whether OS-supplemented formula will model the effect of human milk OS.

Boehm and colleagues [40] examined the effect of OS added to preterm infant formula for a 28-day feeding period. The OS was added at 1g/100ml, with a combination of 90% GOS and 10% FOS. The bifidobacteria counts in the supplemented group significantly increased compared to control, and reached the upper range of the human-milk fed reference group. Stool consistency in the control group was harder and bowel movements occurred less frequently compared to OS group. The same mixture of OS was investigated in term infants at 0.4 g/100 ml and 0.8 g/100 ml to determine dose-related effects (Moro *et al.* 2002). Bifidobacteria had significantly increased in the OS formula groups compared to placebo at day 28, and the effect was dose

dependent. The 0.8 g/100 ml OS group had significantly higher counts of bifidobacteria than the 0.4 g/100 ml group. Lactobacilli also significantly increased in the OS groups compared to control, but there were no differences between the two OS groups. In the 0.8 g/100ml group, fecal pH decreased and stool consistency softened as stool frequency increased. Schmelzle *et al.* [43] conducted a multicenter trial designed to study the efficacy of the addition of prebiotics to infant formula. Over a 12-week period, infants consumed formula supplemented with 0.8 g/100 ml OS, comprising 90% GOS and 10% FOS. The percentage of fecal bifidobacteria had significantly increased in the experimental formula groups at 6 weeks, compared with no change in the control group, and the experimental groups had softer stools at 6 and 10 weeks of life.

A large multicenter growth and safety trial was conducted to evaluate FOS-supplemented infant formula [47]. For 12 weeks, infants ≤ 14 day of life consumed control formula or the same formula supplemented with 1.5 g/L or 3.0 g/L FOS. Anthropometric data demonstrated adequate growth on all measures over the 12-week period, according to the Centers for Disease Control and Prevention reference ranges using percentiles and Z scores. It is of clinical significance that the 3.0 g/L FOS group had fewer adverse events (AEs) than the control group, and the 1.5 g/L FOS group had slightly more AEs than the control group. In particular, the 3.0 g/L FOS group had statistically less constipation in the intent-to-treat population.

REGULATORY ENVIRONMENT

In the European Union (EU) and US, the current legislative and regulatory environments are vague and apparently unsettled with regard to probiotics and prebiotics in infant formula. The addition of microbes to formula is not mentioned in the EU's Infant Formula Directive; microbiological criteria for formula are not harmonized in the EU; and there are no EU regulations on criteria for "probiotic" bacteria or on foods containing them. In early 2002, the Food and Drug Administration (FDA) of the US accepted a Generally Regarded as Safe (GRAS) notice (GRAS Notice No. GRN 000049) from Nestlé USA, for the use of *B. lactis* Bb12 and *S. thermophilus* Th4 as ingredients in formula for healthy infants aged 4 months and older. In its response to Nestlé, the FDA "[had] no questions ... regarding Nestlé's conclusion that *B. lactis* strain Bb12 and *S. thermophilus* strain Th4 are GRAS for their intended use as ingredients in milk-based infant formula that is intended for consumption by infants four months and older at levels not to exceed good manufacturing practice." In a September 2001 report, the EU's Scientific Committee on Food concluded that data was insufficient to establish the safe use of FOS and GOS as infant formula ingredients. Subsequent to this report, several additional studies were submitted and led the Committee to conclude that they had "...no major concerns about [the] combination of [10%] oligofructosyl-saccharose and [90%] oligogalactosyl-lactose in infant formulae and follow-on formulae in total concentrations up to 0.8 g/dL in the product ready for consumption." In the US, the FDA has approved FOS as a GRAS ingredient, but no pre-market notifications have been submitted for infant formula containing oligosaccharides.

CONCLUSION

In the course of the past decade, a number of studies involving the use of probiotics and prebiotics have been conducted in infants. While a variety of specific health outcomes have been examined in these studies, an extremely important result of this collective body of work is the evidence that probiotics and prebiotics are safe and well tolerated in this sensitive population. With respect to gastrointestinal and fecal microbiology, a number of these studies have demonstrated that probiotic microbes ingested by infants can later be recovered, using either cultural or molecular approaches, in feces. However, it is less clear what influence the ingested probiotic(s) has on other important groups of microbes in the gastrointestinal flora; in some instances changes occur, while in other cases, probiotic treatment appears to have little impact on fecal flora populations. Probiotics appear to be a promising modality in the management of infant diarrhea, and a number of well-controlled clinical trials have shown that supplementation of lactobacilli during the acute phase can shorten the duration of diarrhea. Similarly, several studies have demonstrated the value of probiotics in the prevention of diarrhea. Additionally, a number of double-blinded, randomized, and placebo-controlled studies have been conducted to evaluate the onset or severity of atopic disease as a primary endpoint. From these studies, it is apparent that administration of probiotics, especially lactobacilli, to high-risk infants produces a significant improvement in atopic eczema incidence and severity. Finally, several studies have demonstrated that prebiotics may be useful in positively impacting stool attributes of formula-fed infants by altering stool microbiology, pH, and consistency. Prebiotics may also decrease the incidence of constipation.

There is a substantial and evolving body of evidence concerning the safety and efficacy of probiotics and prebiotics in infants. In addition to affirming their safety and utility, future research will be crucial in the evaluation of new strains of probiotics and new varieties of prebiotics (or combinations of these), as well as in the exploration of novel uses in infants. As new data about their use in infants emerge, and as the regulatory climate becomes clearer, the number of products and the use of probiotics and prebiotics are expected to grow.

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